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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 11/00, 11/02, 11/10, 11/08, 11/04, 5/00, A61K 38/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/40889</b> <b>(43) International Publication Date:</b> 19 December 1996 (19.12.96)
<b>(21) International Application Number:</b> PCT/US96/09708 <b>(22) International Filing Date:</b> 7 June 1996 (07.06.96)  <b>(30) Priority Data:</b> 08/480,875                      7 June 1995 (07.06.95)                      US  <b>(71) Applicant:</b> ST. JUDE MEDICAL, INC. [US/US]; one Lillehei Plaza, St. Paul, MN 55117 (US).  <b>(72) Inventor:</b> CAMPBELL, Todd, D.; 4951 Birch Lake Circle, White Bear Lake, MN 55110 (US).  <b>(74) Agents:</b> ELLINGER, Mark, S.; Fish & Richardson, 3300 Dain Bosworth Plaza, 60 South Sixth Street, Minneapolis, MN 55402 (US) et al.		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHOD FOR SPATIALLY POSITIONING CELLS WITHIN A THREE-DIMENSIONAL MATRIX  <b>(57) Abstract</b>  Disclosed is a method for positioning cells within a three-dimensional matrix. Jet injection technology is used to propel cells at a three-dimensional matrix such that the cells become embedded within the interior of the matrix.		

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METHOD FOR SPATIALLY POSITIONING  
CELLS WITHIN A THREE-DIMENSIONAL MATRIX

Field of the Invention

5           This invention relates to a method for spatially positioning cells within a three-dimensional matrix. More particularly, this invention is directed to jet injection of cells into a tissue-derived or synthetic three-dimensional matrix.

Background of the Invention

10           The field of tissue and organ transplantation is growing rapidly as a result of advances in the areas of organ preservation, surgical techniques, immunosuppression and graft/host interaction. As a  
15 result, lack of supply or ready availability of implantable material is often the major obstacle to use of bioprosthetic implants. Improved methods of cryopreservation have increased the number of available bioprosthetic implants. However, shortages of  
20 implantable materials for human patients are especially acute for heart valves, where autologous structures such as the pulmonic valve can only infrequently be used as a source of replacement material, and allogeneic implantable materials are limited.

25           As used herein, the term "autologous" refers to cells, tissues or other biological structures derived from the same individual designated to receive the implant. The terms "allogeneic" and "homogeneic" refer to cells, tissues or other biological structures taken  
30 from other members of the same species. The term "xenogeneic" refers to cells, tissues or other biological

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structures taken from a member of a species other than the species of the individual receiving the implant.

Prosthetic heart valves, used to replace defective heart valves and other vascular structures, may be classified as mechanical or bioprosthetic. Mechanical heart valves typically have a rigid orifice ring and rigid hinged leaflets, and are manufactured from biocompatible metals and other materials such as Silastic®, graphite, titanium, and Dacron®. Though mechanical valves have established a record of durability over decades of use, they are frequently associated with a high incidence of blood clotting on or around the valve. This can lead to acute or subacute closure. For this reason, patients with implanted mechanical valves must remain on anticoagulants as long as the valve remains implanted (typically for life). Anticoagulants are inconvenient to take and impart on the patient a 3-5% annual risk of significant bleeding and a concomitant change in life style.

Bioprosthetic valves typically include valve leaflets formed of biological material. Bioprosthetic valves were introduced in the early 1960's and can be retrieved from either a deceased human ("homograft") or from a slaughtered pig or other mammal ("xenograft"). The valves are typically derived from pig aortic valves or are manufactured from other biological materials such as bovine pericardium. Xenograft heart valves typically are crosslinked in glutaraldehyde prior to implantation.

All mechanical or bioprosthetic heart valves, although greatly improving the patient's condition, have significant disadvantages. These include thrombogenicity (tendency towards thrombus formation and subsequent detachment with embolization) and limited durability secondary to mechanical or tissue structural failure. Other complications such as noise, hemolysis (destruction

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of blood elements), risk of endocarditis (valve infection) and partial dehiscence of the valve also occur.

A major rationale for the use of autologous,  
5 allogeneic or xenogeneic biological material for heart valves is that the profile and surface characteristics of this material are optimal for laminar, nonturbulent blood flow. As a result, intravascular clotting is less likely to occur than with mechanical valves. This effect has  
10 been proven in clinical use with the well-documented reduced thrombogenicity of current versions of glutaraldehyde-fixed bioprosthetic valves. In addition, mechanical valves typically fail suddenly and without warning, resulting in emergency situations requiring  
15 surgical intervention and replacement of the artificial prosthesis. Bioprosthetic valves, on the other hand, tend to wear out gradually, giving the patient and treating physician advanced warning that a failure is likely.

20 A major disadvantage of bioprosthetic devices is the failure of such devices to be self-maintaining. Generally, neither cadaveric homografts nor glutaraldehyde-fixed xenografts have significant populations of viable cells; glutaraldehyde, for example,  
25 is highly cytotoxic. Since viable cells in the valve provide protection against the insudation of calcium, it is likely that any devitalized bioprosthesis will undergo calcification over time. It is essential to the development of a durable prosthetic device, therefore, to  
30 decellularize the extracellular matrix so that it can support ingrowth and colonization of cells. Various detergents and enzymes have been used in the past to obtain extracellular matrices from body sources for use as potential graft materials.

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The repopulation of the matrix by contiguous cells from the host during the period after implantation, by innoculation of autologous or allogeneic sources in tissue culture before implantation, or from both the host  
5 after implantation and from various sources prior to implantation, can be critical to development of a successful implant. Current recellularization processes, however, are time-consuming and expensive. The primary method used to recellularize three-dimensional  
10 extracellular matrices is to apply the cellular material to the surface of the matrix and to allow for a period of cell multiplication and in-migration. Cells derived from the initially seeded population may, under these conditions, migrate among the collagen and elastin fibers  
15 and become spatially positioned within the matrix. Experience indicates that this process can require extensive time periods, which may not be suitable for manufacturing purposes.

One alternative to recellularization by long-term  
20 culture involves injecting cells into the matrix through a small bore needle. This method is impractical, however, as it requires unacceptable puncturing of the matrix by the needle tip. The method is also not optimal because it typically results in an uneven dispersal of  
25 cells within the matrix.

Moreover, the physical constraints imposed by particular surgical implantation methods may limit the effectiveness of existing recellularization techniques. For example, methods of implantation may include use of a  
30 Dacron® sewing ring for xenograft heart valves, or freehand attachment via the root structure in the case of allogeneic heart valves. Each of these methods presents distinctive challenges for obtaining adequate recellularization of the decellularized matrix.

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Jet injection technology involves application of pressure to propel molecules or particles dissolved or suspended in a liquid with a force adequate to penetrate a target tissue. See, for example, U.S. Patent No. 5,064,413, incorporated by reference herein. Generally, a liquid to be injected (the injectate) is loaded into a chamber having a relatively small injection orifice or nozzle; the chamber is coupled to a pressure-generating apparatus that initiates and sustains the propulsive force for a defined time interval. Jet injection technology has been used clinically since 1947 for successful subcutaneous, sub/intradermal, and intramuscular vaccine injections. Typical injectates have been vaccines or other medicines that have otherwise been delivered by needle and syringe. Early jet injectors, however, received limited use because they were relatively cumbersome and difficult to operate.

The potential for the spread of communicable diseases from needle sticks, and the clinical availability of more operator-friendly injection devices, have renewed interest in this technology. One modern jet injection device is the Biojector® 2000, a product marketed by Bioject Inc., Portland, Oregon (Bioject Inc. Cat.# 1 B02000). This device utilizes modern jet injection technology and various pressure profiles to deliver macromolecular compositions subcutaneously, sub/intradermally or intramuscularly. The Biojector® 2000 uses compressed carbon dioxide (CO<sub>2</sub>) as a power source to push the plunger of a needleless, disposable syringe, expelling an injectate through a micro-orifice within a fraction of a second.

Jet injection technologies have also received attention as possible means for injecting DNA into cells for purposes of genetic engineering. Other technologies similar to jet injection have been used for introduction

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of DNA and other macromolecules into cells or cell structures by accelerating the macromolecule. Examples of these technologies can be found in Crossway, U.S. Pat. No. 4,743,548, and Sanford, U.S. Pat. Nos. 4,945,050, 5,100,792, 5,204,253 and 5,371,015. In general, all of these technologies deal with placement of DNA and other organelle components into the cell through the cell membrane. While many of these implanted proteins are rather large, none of the present approaches utilize the concept of placing intact cells into a three-dimensional tissue-derived matrix for the purpose of pre-seeding the matrix with autologous, allogeneic or xenogeneic cells prior to implantation.

#### Summary of the Invention

The method of the present invention allows for spatially positioning viable cells within a three-dimensional matrix. The method comprises propelling the cells toward the matrix at a velocity sufficient to cause the cells to penetrate the surface of the matrix and to become located within the interior of the matrix. Either before or during the propelling operation, the cells may be positioned relative to the matrix such that the cells are directed to a desired location within the matrix. Such positioning may be controlled in a predetermined manner, for example with computer-driven precision numerical control of the direction of propelling.

The cells may be treated with a coating substance prior to propelling or while propelling the cells at the three-dimensional matrix. The coating substance may comprise, for example, phospholipid. Alternatively, or in addition, the cells may be treated with a cryoprotective substance prior to propelling. The cryoprotective substance penetrates the cell membranes and permeates the cytoplasm, such that the cells become



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invested with the cryoprotective substance. Preferably the cryoprotective substance has a viscosity greater than water. The cryoprotected cells may be in a frozen state when propelled at the three-dimensional matrix.

5       The three-dimensional matrix may comprise any extracellular matrix derived from a body tissue, for example a porcine heart valve. The method of the present invention is also appropriate for a fibrous vascular component of a three-dimensional matrix. Alternatively,  
10 the three-dimensional matrix may comprise a synthetic matrix.

      In a further alternative embodiment, the methods of the present invention are applied to propelling cells in situ, i.e., to propelling cells at appropriate targets  
15 inside the body.

#### Brief Description of the Drawings

Figure 1 is a graphical representation of a standard injection pressure profile.

Figure 2 is a photomicrograph of non-ruptured  
20 cells that were subjected to a jet injection pressure profile.

#### Detailed Description

The present invention provides a method for introducing intact cells into a three-dimensional tissue-  
25 derived matrix. A biological tissue matrix useful in the present invention is typically derived from tissue harvested from a human cadaver or animal donor, for example a pig. The harvested tissue is subjected to a decellularizing process to remove viable antigenic cells  
30 from the structural matrix without damaging the structural integrity of the collagen/elastin matrix. Any protocol for producing a three-dimensional matrix suitable for recellularization and for ultimate use with

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human patients is appropriately used in the present invention. A variety of decellularization procedures may be used to provide a suitable matrix. These can include detergent treatment of glutaraldehyde-fixed body

- 5 structures as disclosed in U.S. Patent No. 4,323,358, incorporated herein by reference. Decellularization of unfixed, untreated body structures by detergent methods is disclosed in U.S. Patent No's. 4,352,887 and 4,801,299, both patents being incorporated herein by reference.
- 10 U.S. Patent No. 4,776,853, incorporated herein by reference, discloses a specific process for achieving decellularization by a combination of nonionic and anionic detergents, deoxyribonuclease and ribonuclease. U.S. Patent Application Serial No. 08/424,218,
- 15 incorporated herein by reference, discloses an enzyme-based method of decellularization.

- A synthetic three-dimensional anatomical matrix also may be employed to approximate a biological cellular matrix. Thus, matrices comprising, for example, meshes
- 20 of biocompatible synthetic materials may be used with the methods of the present invention. Appropriate synthetic materials include, without limitation, nylon (polyamides), polyesters, polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g.,
- 25 polyvinylchloride), polycarbonate (PVC), polytetrafluoroethylene, thermonox, nitrocellulose, and polyglycolic acid. The matrices may be prepared as disclosed, for example, in U.S. Patent Nos. 4,963,489 and 5,266,480, incorporated by reference herein.

- 30 Appropriate primary cells or cell lines are selected for injection into the three-dimensional cellular matrix. Liquid (e.g., cell culture medium or isotonic saline) with suspended cells is loaded into a confined vial. Preferably the cell population is in the
- 35 form of a single-cell suspension, although the presence

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of small cell clumps of two or more cells is not precluded. The liquid with suspended cells is forced through a small orifice under high pressure, with the expelled injectate being directed at a tissue-derived  
5 three-dimensional matrix.

The performance of a jet injector is influenced by the pressure characteristics generated during the firing cycle. The pressure produced by a jet injector over the time the injector fires may be graphically presented as a  
10 pressure profile. An entire firing cycle typically lasts only a small fraction of a second.

Fig. 1 illustrates the form of a standard injection profile. The profile graph depicts injector pressure on the Y-axis versus time on the X-axis. The  
15 pressure rise time 1 refers to the amount of time required for the injector to transition from a steady-state ambient pressure 2 to penetration pressure 3. The injector must be capable of reaching penetration pressure 3 in a very short time. If pressure rise time 1 is too  
20 long, the injectate may be ejected before the injector develops sufficient pressure to propel the injectate to velocity sufficient to penetrate the surface of the targeted matrix.

The penetration pressure duration 4 reflects the  
25 length of time that the injectate is subjected to the penetration pressure 3. The injector must, through the penetration pressure 3, impart sufficient kinetic energy upon the injectate that the injectate breaks through the surface of the targeted matrix. Depending on the matrix  
30 composition, the initial "breakthrough" of the matrix may require more energy than the subsequent delivery of injectate into the matrix; therefore, the injector pressure preferably, but not necessarily, decreases after penetration. The drop time to delivery pressure 5  
35 reflects the length of time required for the injector to

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fall from penetration pressure 3 to delivery pressure 6. It is desirable to make the drop time to delivery pressure 5 as short as possible.

The injector operates at the delivery pressure 6 for the remainder of the firing cycle. The delivery pressure 6 is the injector pressure required to drive the remainder of the injectate into the cellular matrix after penetration has occurred. A higher delivery pressure 6 will drive injectate deeper into the cellular matrix than a lower delivery pressure 6.

The injection profile of any particular injector can be adjusted for a specific application. Variables that affect the desired pressure profile include: the composition of the targeted tissue or tissue matrix, the injectate composition, and the spatial relationship between the injector and the targeted tissue. Adjusting the profile involves altering the magnitudes of the rise time 1, penetration pressure 3, penetration pressure duration 4, drop time to delivery pressure 5, and the delivery pressure 6. In addition, nozzle or orifice geometry and tip geometry may also be varied to correspond to particular characteristics associated with the injectate or target.

Injector adjustment or "tuning" is particularly relevant when operating on cellular matrices of varying density. Tuning of the injector permits the injectate to be successfully injected into different cellular matrices. When the injectate is viable cells, tuning permits the cells to be injected without rupture or undue damage. For example, injector tuning permits injection of cells into both the thick fibrous vascular regions and the thin filamentous collagenous leaflet regions of valvular tissue implants.

Pneumatic jet injection technology additionally may be used in a production environment for the

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manufacture of cellular devices. A computer numerically controlled apparatus may be used to position the injection device or injection nozzle in close proximity to the three-dimensional matrix. A numerical program can be employed to inject a predetermined amount of cellular injectate with a programmed velocity at a three-dimensional matrix. This effects a controlled matrix penetration for spatially positioning the cellular injectate in the desired location within the matrix. The program can also control the movement of the injector in a preprogrammed manner in the x, y, and z axes, and can control rotation of the injector or nozzle to permit complete uniform application of cellular injectate throughout the entire structure of the three-dimensional matrix.

It is possible, with xenogeneic, allogeneic or host-derived tissue valve or graft matrix, to use precision numerical control to place the cellular injectate in a relatively precise desired pattern, such as within or around the coronary sinus regions of a heart valve, or in an even distribution within the circumference of a vascular graft. By using numerical control and device-specific injectors, it is further possible to direct the cellular injectate toward either the adventitial or luminal surfaces as necessary, depending on the properties of the matrix and the desired spatial arrangement of the cells.

The numerical controlled process is capable of achieving reproducible results under exacting specifications. Machine vision or many other well-known automated production techniques can be used to adapt each injection process to the dimensions of the particular prosthesis. In addition, injection profiles and injectate placement programs can be stored on various

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electronic media for use with automated injection machinery.

Moreover, jet injection technology may be adapted to deliver intact cells in situ. A Biojector® 2000-like  
5 device may be configured to deliver cells at high velocity into an endoscopy apparatus for targeted delivery to a selected location within the body. Alternatively, with miniaturization techniques, a jet injection device may be fashioned to be used at the  
10 terminus of an endoscopy-like device for in situ delivery of cells. In this manner, cells may be delivered to selected internal locations in the body for various purposes. For example, genetically engineered endothelial or smooth muscle cells may be delivered to a  
15 region of a blood vessel at risk for re-stenosis following an angioplasty procedure.

In an alternative embodiment, the cells to be jet injected into a matrix are pretreated with a coating substance, for example phospholipid. This coating acts  
20 to absorb the shear forces acting on the plasma membranes, and may be "sacrificed" by being sloughed off as the cell passes into the matrix. Thus, by sacrificially lubricating the cells, the coating reduces the shear forces between the cell membranes and the  
25 extracellular materials as the cells enter the matrix. As a result, the cell membranes are subjected to less stress and are less likely to rupture or to receive other shear-related damage.

In a further alternative embodiment, the cells to  
30 be implanted are treated with one or more cryoprotective or functionally similar agents. Particularly for cryoprotectants having a viscosity greater than water (e.g., glycerol), investment of the cells with the cryoprotectant prevents severe displacements of  
35 cytoplasmic and nuclear constituents during acceleration

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and deceleration. As such, the cryoprotectants operate to retard this movement, thereby protecting the cells from rupture or internal damage. In a further embodiment, the cryoprotectant-treated cells may be  
5 frozen and jet-injected as frozen particles into the tissue-derived matrix. The cells may be frozen and accelerated using procedures similar to those disclosed in U.S. Patent No. 5,219,746, incorporated by reference herein. Alternatively, a Biojector® 2000-like device may  
10 be adapted to accelerate frozen particles including frozen entrapped cells. Upon lodgement within the matrix, the cells undergo rapid thawing and resume cellular activity.

In a preferred embodiment, non-frozen cells to be  
15 injected into a matrix are both coated with a shear-protecting layer such as phospholipid, and are invested with a cryoprotectant such as described above. This provides both surface and internal protection to the cells during injection.

20 Example 1

Human dermal fibroblasts obtained from neonatal foreskin by dissociation were fixed in 10% formalin. Then, cells were suspended ( $10^6/\text{ml}$ ) in Dulbecco's Minimal Essential Medium (DMEM) at room temperature and placed  
25 into a Bioject Biojector® 2000 syringe. The suspended cells were subsequently subjected to a subcutaneous jet injection profile including a maximum pressure of 4000 p.s.i. using a Bioject Biojector® 2000 handpiece. The syringe nozzle with micro-orifice was placed at the mouth  
30 of a standard 10 cc glass test tube and the injectate was directed at the glass surface at the opposite end of the tube. After injection, the cells were grossly examined under low power magnification (approximately 10x) in the test tube.

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Histological slides were then prepared by removing the cell suspension from the glass test tube, placing it on a glass microscope slide, air drying the suspension, and staining it with hematoxylin and eosin (H&E). The prepared slides were examined under a microscope. A representative micrograph in Fig. 2 shows the stained cells after injection. The histological analysis revealed the cells to be substantially intact.

#### Example 2

Human dermal fibroblasts are obtained as described in Example 1, above. Porcine aortic root samples are extracted and decellularized with an enzymatic process as described in U.S. Patent Application Serial No. 08/424,218. Then,  $10^6$  cells are suspended in DMEM at room temperature and placed into a Bioject, Inc. Biojector® 2000 syringe or equivalent thereof. The cells in suspension are subsequently subjected to a programmed jet injection profile using a Biojector® 2000 handpiece or equivalent. The cell suspension is injected into a standard laboratory glass test tube. Another cell suspension is injected into the decellularized porcine aortic root samples. For purposes of orientation during injection, the aortic root samples are placed on a mandrel selected to approximate the diameter of the valve orifice. The root samples are kept moist with saline or DMEM.

Histological slides of the cell suspension injected into the glass test tube are prepared by placing the cell suspension onto a glass microscope slide, air drying the suspension, and staining with H&E. Examination of the stained cells demonstrates that the cells are able to withstand exposure to various jet injection profiles.



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Histological slides of the decellularized and jet-injected aortic root are prepared by post fixing the samples in formalin, embedding in paraffin, sectioning at  $10\mu$ , and staining with H&E.

5

Example 3

Human dermal fibroblasts are obtained as described above in Example 1. The cells are preconditioned by exposing the cells to a solution of cryoprotectant (10% DMSO or 10% glycerol) in DMEM. Some of these cells are also coated with a layer of phospholipid. Porcine aortic root samples are extracted and decellularized with an enzymatic process as described in U.S. Patent Application Serial No. 08/424,218. Then,  $10^6$  cells, either phospholipid-coated or exposed to cryoprotectant, or both, are suspended in DMEM at room temperature and placed into a Bioject Biojector® 2000 syringe or equivalent thereof. The cells in suspension are subsequently subjected to a selected programmed jet injection profile using a Biojector® 2000 handpiece or equivalent. The cell suspension is injected into a standard laboratory glass test tube. Another cell suspension is injected into the decellularized porcine aortic root samples as described above in Example 2.

Histological slides of the cell suspension injected into the glass test tube are prepared by placing the cell suspension onto a glass microscope slide, air drying the suspension, and staining with H&E. Examination of the stained cells is found to demonstrate that the phospholipid-coated, cryoprotectant-treated, and coated and cryoprotected cells are able to withstand exposure to various jet injection profiles.

Histological slides of the decellularized aortic root are prepared by post fixing the samples in formalin,

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embedding them in paraffin, cutting 10 $\mu$  sections, and staining with hematoxylin and eosin.

The foregoing detailed description has been provided for a better understanding of the invention only  
5 and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those skilled in the art without deviating from the spirit and scope of the appended claims.

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What is claimed is:

1. A method for spatially positioning viable cells within a three-dimensional matrix, comprising propelling said cells toward said matrix at a velocity sufficient to,  
5 cause said cells to penetrate the surface of said matrix and to become located within the interior of said matrix.
2. The method of claim 1, further comprising positioning said cells relative to said matrix prior to propelling or while propelling said cells at said matrix.
- 10 3. The method of claim 2, wherein said positioning is controlled in a predetermined manner.
4. The method of claim 1, wherein said cells are treated with a coating substance prior to propelling or while propelling said cells at said three-dimensional  
15 matrix.
5. The method of claim 4, wherein said coating substance comprises phospholipid.
6. The method of claim 1, wherein said cells are invested with a cryprotective substance prior to said  
20 propelling.
7. The method of claim 1, wherein said cells are frozen.
8. The method of claim 1, wherein said three-dimensional matrix comprises an extracellular matrix  
25 derived from a porcine heart valve.

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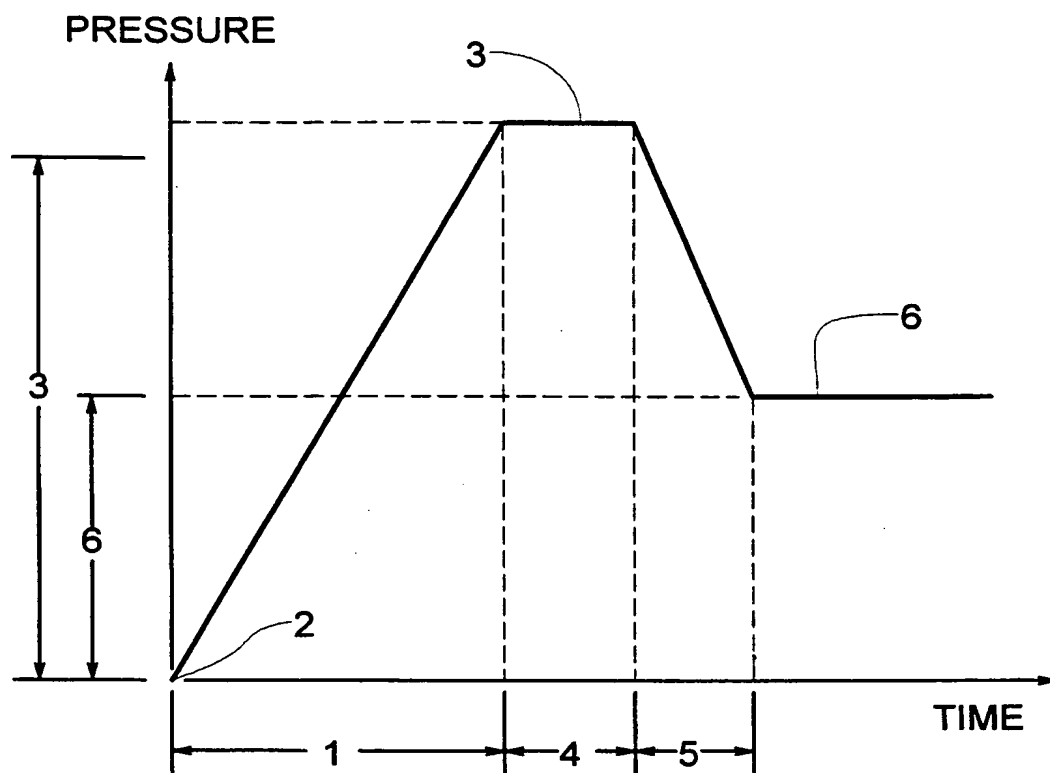
9. The method of claim 8, wherein said three-dimensional matrix comprises a fibrous vascular component.

10. The method of claim 1, wherein said three-  
5 dimensional matrix comprises a synthetic matrix.

11. The method of claim 1 wherein said propelling is conducted in situ.

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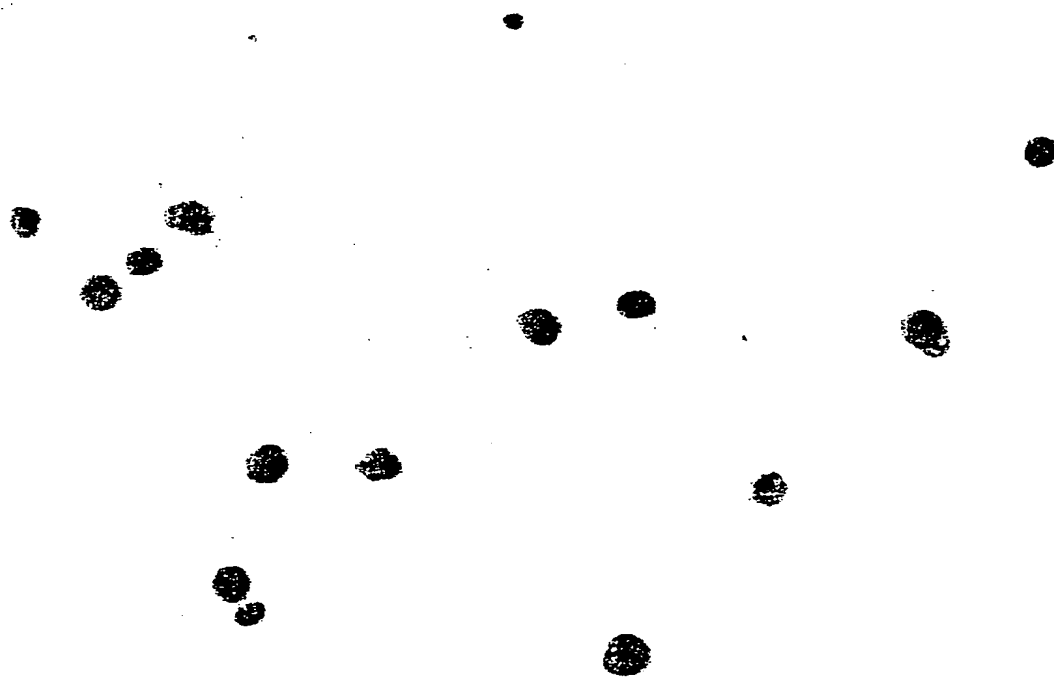
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*Fig. 1*

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**Fig. 2**



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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/09708

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 11/00, 11/02, 11/10, 11/08, 11/04, 5/00; A61K 38/00

US CL : 435/174, 177, 178, 180, 182, 240.23; 424/93.7

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/174, 177, 178, 180, 182, 240.23; 424/93.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: three-dimensional matrix, propelling cells, jet injection, spatially positioning cells

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,505,266 A (YANNAS ET AL) 19 March 1985 (19.03.85), entire document.	1-11
Y	US 5,219,746 A (BRINEGAR ET AL) 15 June 1993 (15.06.93), entire document.	1-11
Y	US 4,963,489 A (NAUGHTON ET AL) 16 October 1990 (16.10.90), entire document.	1-11
Y	DAVIS et al. Direct Gene Transfer in Skeletal Muscle: Plasmid DNA-Based Immunization Against the Hepatitis B Virus Surface Antigen. Vaccine. December 1994, Vol. 12, No. 16, pages 1503-1509, see entire document.	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

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